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# Application of Haemagglutination Inhibition Tests in the Follow Up of Choriocarcinoma: A Comparative Study

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The sequential course of urinary choriogonadotropin (HCG) levels was studied by commercial haemagglutination inhibition tests in three patients suffering from chorionepithelioma. The data were correlated with bioassays and the clinical course of the disease. The test were found to be clinically useful during treatment and follow up. No indications were found that the drugs used interfered with the HCG assays.

Bei drei an Chorionepitheliom erkrankten Patientinnen wurde der zeitliche Verlauf der Choriongonadotropin (HCG)-Ausscheidung im Harn mit käuflichen Hämagglutinations-Hemmtests verfolgt. Die Werte wurden mit den Ergebnissen biologischer HCG-Tests verglichen und mit dem klinischen Verlauf der Erkrankung in Beziehung gesetzt. Die Tests erwiesen sich als klinisch brauchbar für die Kontrolle der HCG-Ausscheidung unter der Behandlung. Für eine Störung der HCG-Bestimmungen durch die den Patientinnen verabreichten Medikamente ergab sich kein Anhalt.

For the diagnosis and control of the treatment of placental choriocarcinoma a knowledge of the urinary excretion of human chorionic gonadotropin (HCG) is indispensable (1).

Several methods for assaying HCG from urine have been described, including the techniques of bioassay, radio-immunoassay, or the haemagglutination inhibition test. Our bioassay is susceptible to subjective interpretation and it suffers from the other disadvantage of inadequate sensitivity to low levels of HCG. The radio-immuno-assay has the attractive advantage of giving an absolute value for HCG. It demands, however, a big outlay in money and equipment, and like the bioassay it takes several days to obtain results (2, 3). Test kits for the haemagglutination inhibition test of HCG, containing sheep erythrocytes coated with HCG and the antiserum to HCG are commercially available.

We have used two commercial tests (Pregnosticon and Luteonosticon) which provide results within 2 and 8 hours respectively. The tests are simple to perform. The former is designed as a pregnancy test and is a direct test for HCG. The Luteonosticon test has been developed primarily for the assay of LH by making use of the cross reactivity between antiserum against HCG and LH. This implies that in the very low ranges, LH rather than HCG is expected to be measured. Whereas the Pregnosticon test requires urine containing more than 1500 IU/l for a positive result, the Luteonosticon test is capable of detecting 10 IU HCG (or 25 IU LH) per liter urine. This means that nearly the whole range of HCG excretion levels to be expected in patients suffering from choriocarcinoma can be covered by using both tests. Only in cases of very low HCG titers does the lack of discrimination between LH and HCG prevent a correct interpretation of the assay result in terms of HCG. The present paper describes the sequential course of HCG excretions from patients suffering from choriocarcinoma. Both tests, Pregnosticon and Luteonosticon, were used throughout the follow up, depending on the amount of HCG excreted. The results are related to the clinical data of the patients. For comparative purposes, several urine samples were tested for HCG by bioassay.

## Methods and Materials

### Haemagglutination inhibition test

The HCG assays were performed with the haemagglutination inhibition tests Pregnosticon All-In and Luteonosticon (Organon, Oss, the Netherlands).

#### Principle of the test

Erythrocytes coated with HCG retain their capacity to settle in a ring formation, provided tubes with a hemispherical bottom are used.

The presence of antibodies to HCG will inhibit this ring formation. If a sample contains HCG sufficient to block the action of the antibodies the formation of a ring pattern will therefore be uninhibited (4, 5, 6).

Pregnosticon All-In is standardized on a level of 1500 IU HCG/l urine, using the 2nd International Standard for HCG as a standard preparation.

Luteonosticon is prepared using carefully chosen antisera which possess such cross reactivity to LH that 2.5 IU LH show a haemagglutination inhibition activity comparable to that of 1 IU of HCG. As Luteonosticon is standardized on a level of 25 IU LH/l urine (2nd IRP — HMG) this can be 'translated' into 10 IU of HCG.

#### Procedures for the Pregnosticon and Luteonosticon tests

Twenty four hour urine samples were collected, in which assays for creatinine were performed to control complete collection. Urine samples were kept frozen unless assayed immediately. Freezing and thawing, even when done twice, did not affect the results. After thawing completely the sample was well mixed and centrifuged. Dilutions were made up with 0.1% bovine serum

albumin solution in physiological saline to prevent adsorption of HCG or LH to the glass wall.
There are two points which, if not considered properly, may lead to erroneous results. All reagents used must belong to the same batch. Secondly, any vibration during the formation of the sedimentation patterns should be avoided.

### Dilution scheme

Serial dilution of a sample allows one to estimate, from the sedimentation pattern, the upper and lower limits of the range in which true HCG concentration exists. The highest dilution still giving an agglutination inhibition (ring formation) is taken for the calculation of the lower limit of the range. For the calculation of the upper limit the lowest dilution is used, still giving an intermediate pattern, or a diffuse sedimentation. Thus the number of dilutions, and the extent to which they are performed, determine the limits of these ranges. In selecting a scheme for dilutions one must compromise. The desire to express the results in narrow ranges conflicts with the demands of practice and economy. As a compromise, a schedule for the dilution series has been set up which allows the results to be expressed in narrow ranges for low levels, but in more expanded ranges as levels tend to become higher.
As soon as HCG excretions fall into the range of normal values for LH, therapeutic treatment has to be continued for some period to eliminate remaining small aggregations of tumour cells (1). During this period the levels of HCG excretions have to be estimated within narrow ranges to detect any recurrence of the disease. Therefore, the above mentioned schedule meets the diagnostic requirements. Since the schedule used in this study deviates from the one given by the manufacturers of the tests the full dilution scheme will be presented below:

*Samples containing 7,000—30,000 IU/l HCG*

Assays were performed according to the procedure described in the leaflet delivered with each Pregnosticon test, but with a modification: To the opened ampoules, numbered 1, 2 and 3, 0.1 ml urine diluted 1:5, 1:10 and 1:20 respectively was added. Thereafter the instruction leaflet was followed. Test results can be read after a period of two hours. A complete agglutination inhibition is revealed by a clear ring formation. The minimum amount of HCG necessary for ring formation is 0.15 IU. Considering the highest dilution (a) which still gives a ring, the minimum amount of HCG to be expected in the urine can be calculated by

$$0.15 \times 10{,}000 \times \text{dilution factor a} \quad \text{(eq. 1)}$$

The result of equation (1) represents the lower limit of the HCG range. The next ampoule in the dilution series (b) will show either no ring or a diffused transitional pattern.
The urine will therefore contain at most an amount of HCG equal to

$$0.15 \times 10{,}000 \times \text{dilution factor b} \quad \text{(eq. 2)}$$

The result of equation (2) represents the upper limit. Table 1 summarizes the possible results (IU/l).

*Samples containing more than 30,000 IU/l HCG*

To the opened ampoules, numbered 1, 2, 3 and 4 of the Pregnosticon test, 0.1 ml urine diluted 1:50; 1:100; 1:500 and 1:1,000 respectively was added. Otherwise the assays were performed following the instruction leaflet. Table 2 summarizes the possible results as calculated from equations (1) and (2).

Tab. 1

HCG concentration ranges as read from the sedimentation pattern and calculated from equations (1) and (2). The sign + denotes ring formation, the sign —, indistinct or no ring formation (Pregnosticon test)

| Ampoule number | 1 | 2 | 3 | HCG concentration range |
|---|---|---|---|---|
| Dilution | 1:5 | 1:10 | 1:20 | IU/l |
| | + | + | + | > 30,000 |
| | + | + | — | 15,000—30,000 |
| | + | — | — | 7,500—15,000 |
| | — | — | — | < 7,500 |

Tab. 2

HCG concentration ranges as read from the sedimentation pattern and calculated from equations (1) and (2). The sign + denotes ring formation, the sign —, indistinct or no ring formation (Pregnosticon test)

| Ampoule number | 1 | 2 | 3 | 4 | HCG concentration range |
|---|---|---|---|---|---|
| Dilution | 1:50 | 1:100 | 1:500 | 1:1000 | IU/l |
| | + | + | + | + | <1,500000 |
| | + | + | + | — | 750,000—1,500000 |
| | + | + | — | — | 150,000— 750,000 |
| | + | — | — | — | 75,000— 150,000 |
| | — | — | — | — | 30,000— 75,000 |

*Samples containing less than 7,000 IU/l HCG*

As a rule samples from new patients were checked with the method described for samples containing 7,000—30,000 IU/l HCG. If ring formation was absent in all of the ampoules, the urine was assayed with the Luteonosticon test. The sensitivity of this test is mainly due to the following steps: Incubation with the antiserum (2—4 h), and a second incubation period of 30—60 min after adding the sensitized erythrocytes. After centrifuging and washing, the erythrocytes are resuspended in a small volume allowing the sedimentation pattern to be read within two hours. For more details, the reader is referred to ref. l. c. (4), (5) and (6).
The protocol of the Luteonosticon test was followed with modifications:

1. All samples were put in a waterbath at 95°C for 5—10 min. After cooling and centrifuging the urines were diluted 1:20.
To the tubes numbered 1—5 containing the antiserum were added 0.0; 2.0; 4.0; 5.0 and 5.5 ml of diluent. The latter was prepared as prescribed in the instruction leaflet. The contents were made up to 6.5 ml with the diluted sample.
2. If the urine was expected to contain less than 200 IU/l HCG the sample was diluted only 1:5. To the tubes numbered 1, 2 and 3 containing the antiserum (0.5 ml) 0.0; 2.0 and 4.0 ml of diluent were added. The contents were made up to 6.5 ml with the diluted sample.

The test is then adjusted to give a ring formation, if the mixture contains at least 0.06 IU HCG. If therefore, in a series, two successive tubes containing respectively "c" and "d" ml diluted urine are characterized by a positive and a negative result the HCG content can be expressed by the range

$$\left(0.06 \times \frac{1{,}000}{d} - 0{,}06 \times \frac{1{,}000}{c}\right) \times \text{dilution factor} \quad \text{(eq. 3)}$$

Table 3 summarizes possible results as derived from equation (3). The results "higher than 2,400 IU/l" with the Luteonosticon test and "less than 7,000 IU/l" with the Pregnosticon test for a given sample are interpreted solely as 2,400—7,000 IU/l.

### Normal range (expressed in terms of HCG)

The acceptable normal range is derived from the LH levels found in normal premenopausal subjects.
These are less than 50 IU/24 h, except for the ovulatory phase in which LH levels expressed as HCG units can rise to 240 IU/24 h.

### Bioassay

The bioassay was performed by a modification of the Aschheim and Zondek method as described by Boot et al. (7). Briefly, 3—4 week old female mice were given three subcutaneous injections of 0.05 ml urine on day 1 and 2. For this, early morning urine samples, diluted (1:10; 1:100 or 1:1,000) or undiluted were used. Vaginal smears were taken on days 4 and 5. At autopsy on day 5, the presence or absence of corpora lutea was noted, as well as follicle development and uterine or ovarian size.

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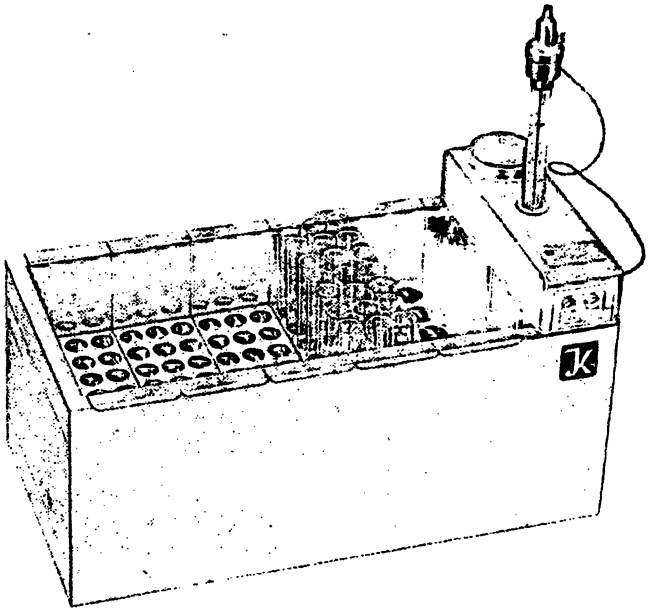

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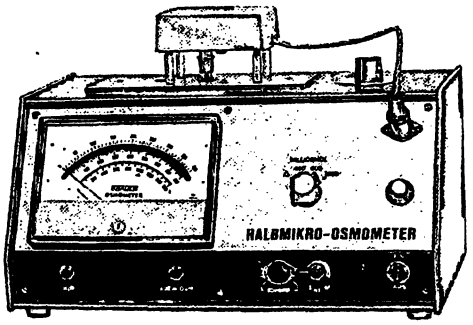


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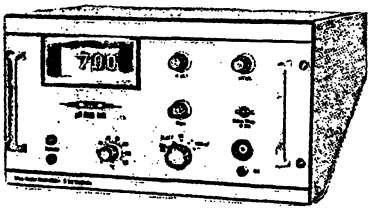


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Tab. 3

HCG concentration ranges as read from the sedimentation pattern and calculated from equation (3). The sign + denotes ring formation, the sign —, indistinct or no ring formation (Luteonosticon test)

| Tube number | 1 | 2 | 3 | 4 | 5 | HCG concentration range |
|---|---|---|---|---|---|---|
| ml diluted urine | 6.0 | 4.0 | 2.0 | 1.0 | 0.5 | IU/l |
| Dilution 1:20 | — | — | — | — | — | < 200 |
| | + | — | — | — | — | 200— 320 |
| | + | + | — | — | — | 320— 600 |
| | + | + | + | — | — | 600—1,200 |
| | + | + | + | + | — | 1,200—2,400 |
| | + | + | + | + | + | > 2,400 |
| ml diluted urine | 6.0 | 4.0 | 2.0 | | | |
| Dilution 1:5 | — | — | — | | | < 50 |
| | + | — | — | | | 50— 75 |
| | + | + | — | | | 75— 150 |
| | + | + | + | | | > 150 |

The limits of detectability are: less than 100 IU/l; 100—1,000; 1,000—10,000 and $10^4$—$10^5$ IU/l.

### Construction of graphs

Upper and lower limits of the concentration ranges as obtained by haemagglutination inhibition tests were multiplied by the 24 h urine volume (litres). The limits obtained by the bioassay are expressed as IU/litre. All values are plotted on a logarithmic scale. The lower limits of detectability are 50 IU/l and 100 IU/l for the Luteonosticon test (using the dilution scheme presented here) and the bioassay respectively.

Negative results with these tests will therefore be expressed in the figures by a single dash or circle on the level of 50 and 100 IU/24 h regardless of the 24 hour volume.

### Clinical Studies

Two patients are shown in which the treatment was not effective. This was, presumably, because of the long delay before the diagnosis was made. In one patient the diagnosis was made early and the result of the treatment was as favourable as might be expected.

*Case No. 1*

This concerns a woman of 26 years. Since May 1971 she experienced irregular vaginal blood loss; in September on gynaecological examination a tumour was seen near the ostium externum of the uterine cervix.

The tumour was resected. Histology: choriocarcinoma. There were no signs of lung metastases. The patient was then referred to our clinic. Pelvic arteriography showed no pathology. Treatment was started immediately. The further course of the disease during treatment is illustrated in figure 1. After HCG excretions had reached consistently normal range three additional courses of chemotherapy were given.

*Case No. 2*

refers to a woman of 29 years. After the birth of her second child (June 1970) she experienced irregular blood loss. Diagnosis of choriocarcinoma was made in July 1971 on a biopsy of vaginal metastases. The patient was then referred to our clinic. Multiple lung metastases were found. Standard treatment with methotrexate and actinomycin D had no effect.

The further course of the disease during treatment is illustrated in figure 2. The patient died of progressive lung metastases in December 1971.

*Case No. 3*

concerns a woman who had her only child in 1967. She had abortions in 1968 and 1970. In June 1971 after six months of irregular blood loss, a vaginal tumour was found. A biopsy from this tumour was diagnosed as being "suspect". Two months later a second biopsy was taken; histological diagnosis: choriocarcinoma. In September 1971, the patient was referred to our clinic.

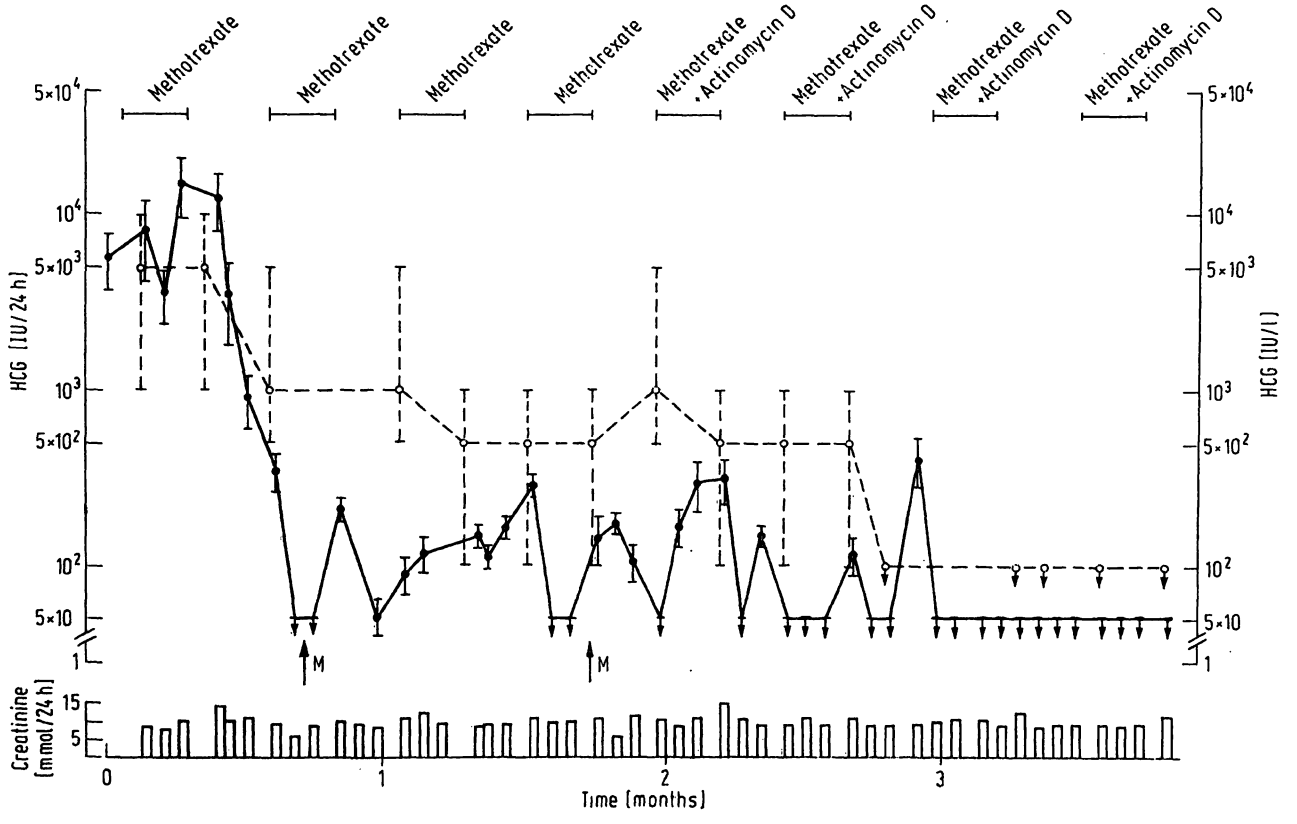


Fig. 1

*Case no 1.* Variation of HCG excretions with time, as measured by haemagglutination inhibition test ([IU/24 h] •—•) and by bioassay ([IU/l] o—o). The upper and lower dash represent upper and lower limits of detectability. The sign M denotes time of menstruation. Chemotherapy is indicated on the head of the graph. Time zero is 16 Sept. 1971. The prints with arrows indicate the detection limit

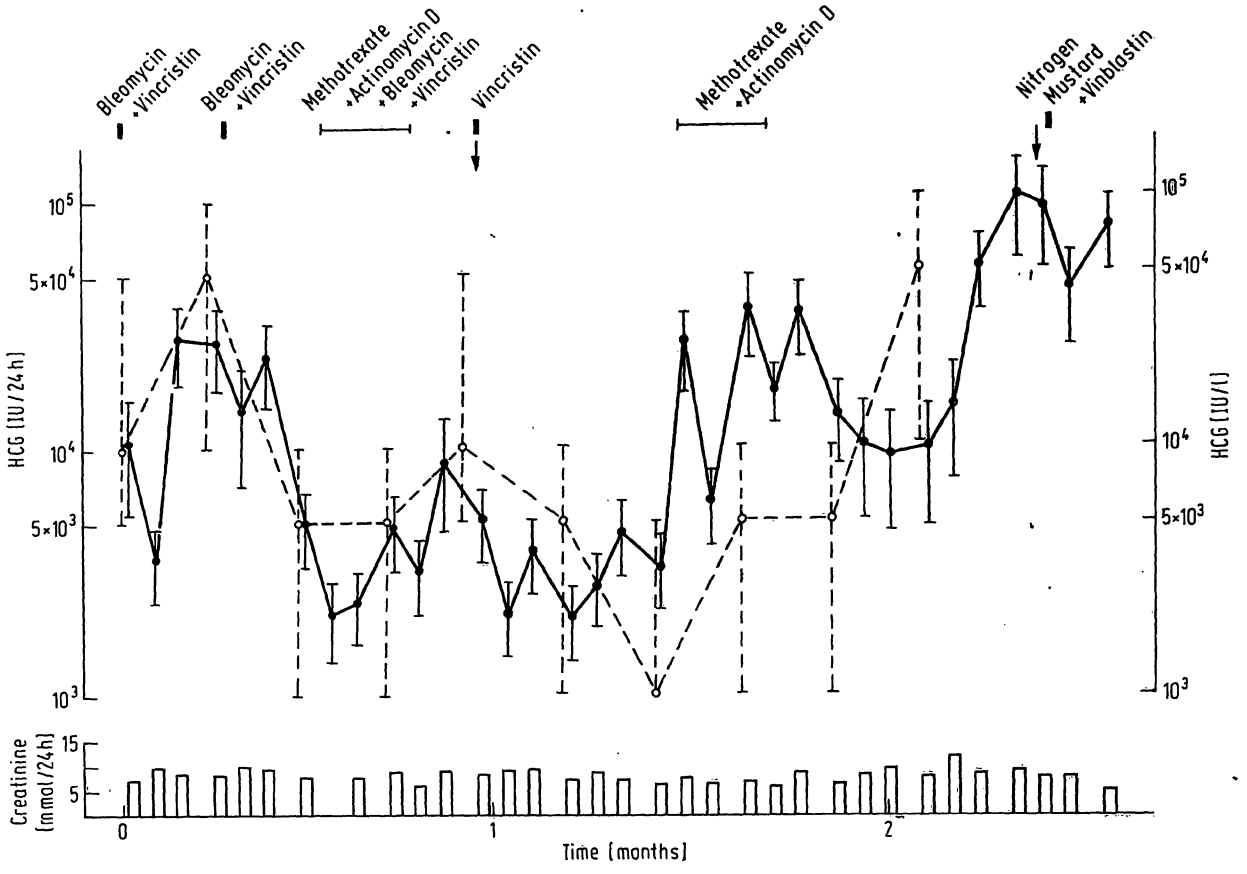


Fig. 2

*Case no 2.* Variation of HCG excretions with time, as measured by haemagglutination inhibition test ([IU/24 h] •—•) and by bioassay ([IU/l] o—o). The upper and lower dash represent upper and lower limits of detectability, chemotherapy is indicated on the head of the graph. Time zero is 26 Sept. 1971

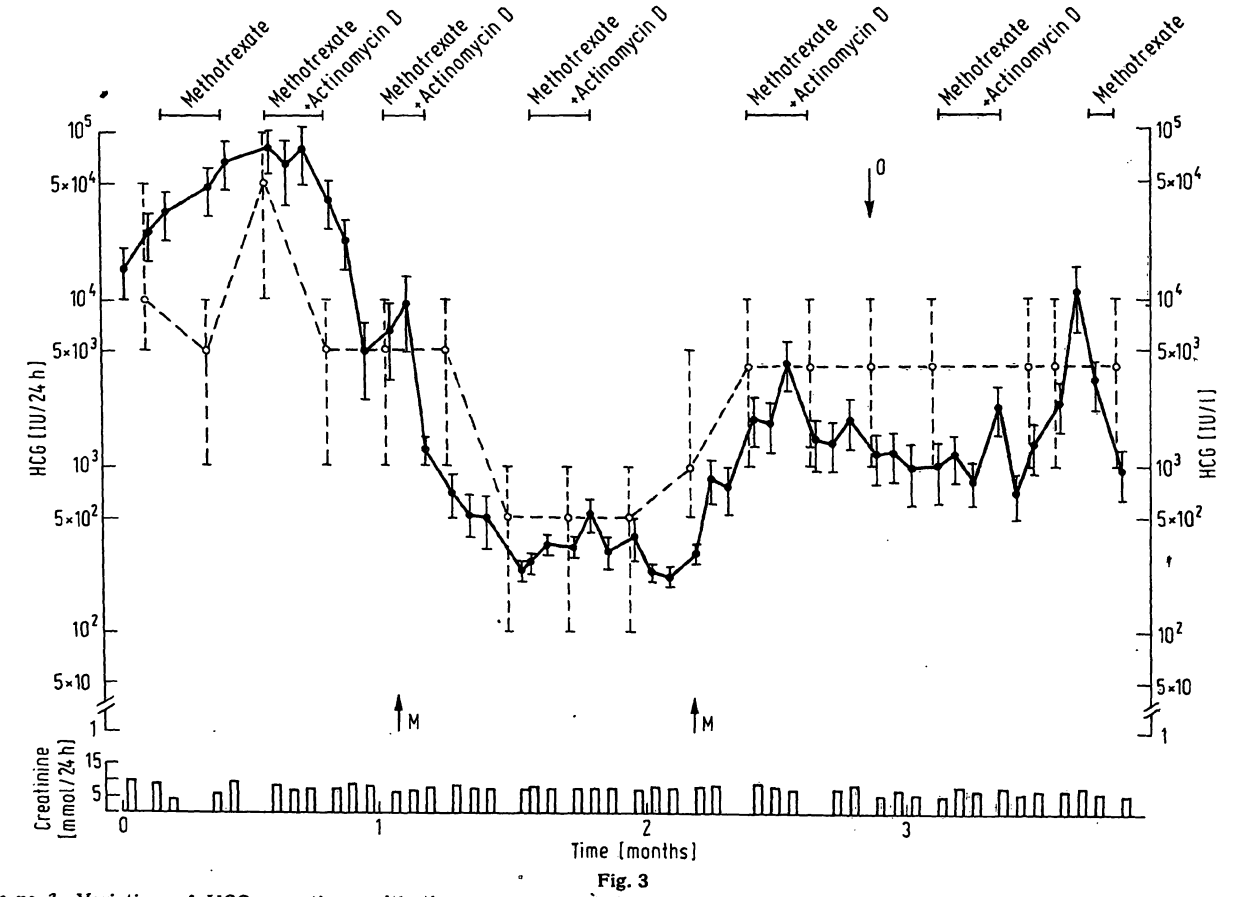


Fig. 3

*Case no 3.* Variation of HCG excretions with time, as measured by haemagglutination inhibition test ([IU/24 h] •—•) and bioassay ([IU/l] o—o). The upper and lower dash represent upper and lower limits of detectability. Chemotherapy is indicated on the head of the graph. The sign O denotes time of operation, the sign M start of the menstruation. Time zero is 10. Sept. 1971

Tab. 4

Recovery experiments using urine samples from patients subjected to different kinds of therapy
P = Pregnosticon test; L = Luteonosticon test

| Experiment No. | Sample | Therapy | Found (IU/l) | Calculated (IU/l) | Test |
|---|---|---|---|---|---|
| 1 | A | none | 30,000— 75,000 | — | P |
| | B | none | <50 | — | L |
| | 1 vol A + 2 vol B | | 7,500— 15,000 | 10,000—25,000 | P |
| 2 | A | none | 600— 1,200 | — | L |
| | B | actinomycin-D and méthotrexate | <50 | — | L |
| | 1 vol A + 5 vol B | | 75— 150 | 100— 200 | L |
| 3 | A | méthotrexate | 200— 320 | — | L |
| | B | actinomycin-D | <50 | — | L |
| | 1 vol A + 3 vol B | | 75— 150 | 50— 110 | L |
| 4 | A | none | 30,000— 75,000 | — | P |
| | B | none | 600— 1,200 | — | L |
| | 1 vol A + 4 vol B | | 7,000— 15,000 | 6,000—16,000 | P |
| 5 | A | N-mustard and vinblastin | 75,000—150,000 | — | P |
| | B | actinomycin-D | 15,000— 30,000 | — | P |
| | 1 vol A + 2 vol B | | 30,000— 75,000 | 35,000—70,000 | P |
| 6 | A | bleiomycin and actinomycin-D | 7,500— 15,000 | — | P |
| | B | actinomycin-D | 200— 320 | — | L |
| | 1 vol A + 6 vol B | | 1,200— 2,400 | 1,240— 2,400 | L |
| 7 | A | N-mustard and vinblastin | 75,000—150,000 | — | P |
| | B | methotrexate | 1,200— 2,400 | — | L |
| | 1 vol A + 11 vol B | | 7,500— 15,000 | 7,300—14,500 | P |
| 8 | A | bleiomycin and actinomycin-D | 7,500— 15,000 | — | P |
| | B | methotrexate | 75— 150 | — | L |
| | 1 vol A + 5 vol B | | 1,200— 2,400 | 1,300— 2,600 | L |
| 9 | A | methotrexate, actinomycin-D and bleiomycin | 1,200— 2,400 | — | L |
| | B | none | <50 | — | L |
| | 1 vol A + 15 vol B | | 75— 150 | 75— 150 | L |
| 10 | A | actinomycin-D and bleiomycin | 7,500— 15,000 | — | P |
| | B | none | <50 | — | L |
| | 1 vol A + 6 vol B | | 1,200— 2,400 | 1,250— 2,500 | L |

X-ray showed multiple lung metastases and on arteriography, indications of pelvic metastases were found. Chemotherapy was started with two courses of methotrexate. The further course of the disease during treatment is illustrated in figure 3.

## Results

Recovery experiments were performed to determine whether acceptable continuity existed between both tests: Pregnosticon and Luteonosticon. Table 4 contains results from these experiments, in which both original urine samples and mixtures of these were assayed for HCG content. For the analyses, tests with different batch numbers were used. The agreement between calculated and observed ranges is satisfactory. Figures 1—3 show the urinary excretions of HCG as determined with the tests and bioassays. In figure 1, there is a good agreement between the results of both assays, especially at the beginning and at the end of the treatment. For unknown reasons, the bioassay shows slightly elevated levels of HCG during a period (2nd and 3rd months in fig. 1) in which the haemagglutination inhibition tests give periodically negative results. For the bioassay early morning urine samples were used. It seems reasonable to assume that the early morning urine, by its higher concentration of HCG as compared to the 24 hours sample, constitutes a borderline case, which is measured as elevated. The periods with continuously negative results with both types of assay, co-incide completely. These tests remained negative for some more weeks (not indicated in the figure). Figure 2 illustrates only part of the clinical course of case No. 2. Initial standard treatments with methotrexate and actinomycin-D had no effect.

Vaginal metastases had regressed significantly at the time indicated by the left arrow. Chest X rays, however, showed that pulmonary metastases did not

regress, but in fact started to increase about three months after the onset of therapy. Clinical signs of pulmonary insufficiency were observed from the time indicated by the right arrow. The patient died one week later (December 1971). The figure shows (left) the temporary effect of the therapy and (right) the increase of HCG levels which corresponded with progressive disease.

Figure 3 (case No. 3) shows good agreement between both assays. There was some regression of the lung metastases and the gonadotropin excretions went down. Hysterectomy and ovariectomy was performed at the time, as indicated by the arrow, because of the progressive nature of the disease. Pelvic arteriography had shown the existence of tumour masses in the uterus and the parametria.

Lung metastases and HCG excretions remained stationary for about three weeks only to become progressive thereafter.

## Discussion

The cells of trophoblastic choriocarcinoma retain their ability to produce gonadotropins. The levels of HCG excretions are more or less representative of the number of tumour cells. The prognosis of choriocarcinoma can be very favourable if the disease is diagnosed early and treated adequately. The latter means that when the tumour responds to chemotherapy and, consequently, HCG excretions have reached normal levels, still two or more courses of chemotherapy must be given to destroy residual tumour tissue. BAGSHAWE (1) has pointed out that in a patient whose HCG excretion has become normal there could be up to $10^5$ tumour cells present. Therefore, continuation of therapy 'to kill the last tumour cell' (BAGSHAWE) is a pre-requisite to prevent a relapse.

From the above, it is of the utmost importance to estimate correctly the beginning of the phase of consistently negative HCG excretions (i. e. equal to the expected LH levels). From figure 1, it is evident that for this purpose a multitude of frequently performed consecutive HCG determinations are necessary. In this figure the normal range is outlined as less than 50 IU. There are two well-known instances where, as a consequence of LH production, values higher than 50 IU/24 h must be taken as the limit of the normal range. During the ovulatory phase there is a short period of elevated LH production, which may result in excretion levels up to 240 IU/24 h (HCG units), and which, obviously, has to be considered in the interpretation of the results. Secondly, ovariectomy disturbs the feed back mechanism of ovarian hormones on the excretion of LH. According to ERB and RICHTER (8) and DEMOL et al. (9) the increase of urinary LH after ovariectomy may vary from 50 IU/24 h to about 500 IU/24 h. This individual variation makes it necessary to establish the normal range for ovariectomized patients separately.

Tab. 5

Comparison of results (IU/l) of HCG analyses by haemagglutination inhibition test with bioassay and radioimmunoassay

| Sample | Haemagglutination inhibition test | Bioassay | Radioimmunoassay |
|---|---|---|---|
| 1 | <50 | <100 | 31 |
| 2 | <50 | — | 42 |
| 3 | <50 | <100 | 83 |
| 4 | 75—150 | 100—1,000 | 120 |
| 5 | 75—150 | 100—1,000 | 81 |
| 6 | <50 | <100 | 30 |
| 7 | 75—150 | 100—1,000 | 40 |

The temporary rise in HCG excretion, which is sometimes seen during and after the first successful courses of therapy, and which may be ascribed to the liberation of HCG from cells killed by chemotherapy, need not lead to interpretation problems.

A question which has not received much attention in the literature is whether drugs (or their metabolites) present in the urine may influence the assay of HCG. It seems that the results of recovery experiments (table 4) strongly argue against any influence. Additional support can be found in the course of the results of the haemagglutination inhibition tests, as compared with those from bioassays: the latter being based on entirely different principles. In a few cases there is a discrepancy, in the sense of a brief opposite effect to the findings of the bioassay and the haemagglutination inhibition tests (see fig. 1 and 3). There is no indication that such a discrepancy is related to the drug applied.

We feel that the haemagglutination inhibition tests can be used for management of chorio-carcinoma. Since, however, in this disease so much depends on the reliability of HCG determinations, we would like to comment on the control of the assay. Regular checks of the HCG analyses with another type of assay (preferably a radio-immunoassay), seems mandatory, especially in the lower ranges. Table 5 gives some examples of checks of results of HCG analyses obtained by haemagglutination inhibition tests, with a bioassay and a radio-immunoassay.

No standard is delivered with the commercial tests used in this study. As an additional control we recommend the preparation of a combined urine sample, containing 200—300 IU HCG/l, which should be divided into equal amounts and stored deep frozen. One portion should be run together with the routine sample as an additional control.

By choice: the bioassay which calls for a great deal of experience does not seem preferable. The introduction of radio-immunoassays in the clinical laboratory becomes attractive if a regular supply of a large number of specimens is expected for a period of years. Even under these circumstances it will be of importance to have haemagglutination inhibition tests at hand which are simple in performance. Radio-immunoassays are known

to fail sometimes from one day to another. To obtain, during the check of the failure of the radio-immunoassay, an uninterrupted delivery of useful HCG data to the clinic one can easily rely on haemagglutination inhibition tests, like those described in this paper.

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## References

1. Bagshawe, K. D., Choriocarcinoma, Edward Arnold Publishers, London (1969). — 2. Midgley, A. R., Endocrinology *79*, 10 (1966). — 3. Bagshawe, K. D., C. E. Wilde and A. H. Orr, Lancet London I/1966, 1118. — 4. Schuurs, A. H. W. M., Acta endocr., K'hvn. *63* (suppl. 142), 95 (1969). — 5. Schuurs, A. H. W. M., J. Endocr. *43*, XX (1969). — 6. Schuurs, A. H. W. M., and C. J. van Wijngaarden, Acta endocrin., K'hvn. *63* (suppl. 141), 13 (1969). — 7. Boot, L. M., M. Frenkel and I. S. Sindram, Nederl. Tijdschr. Verloskunde *66*, 24 (1966) (in English). — 8. Erb, H. and R. H. H. Richter, Acta endocr., K'hvn. *63*, (suppl. 141), 119 (1969). — 9. Demol, R., D. Vandekerckhove and R. Hooghe, Acta endocr. K'hvn. *63* (suppl. 141), 111 (1969).

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